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“A MICROFLUIDIC DEVICE TO DETERMINE DIELECTRIC PROPERTIES OF A SINGLE CELL: A COMBINED DIELECTROPHORESIS AND ELECTROROTATION TECHNIQUE”

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KEYWORDS

Electrorotation, Electrokinetic, dielectrophoresis, cell, microfluidics.

ABSTRACT

Electric fields interaction with living cells is commonly used in lab-on-chips. Indeed AC electrokinetic techniques (dielectrophoresis, electrorotation and traveling wave dielectrophoresis) are used to handle, trap or separate biological entities (eukaryotic cells, bacteria, yeasts, algae) in microfluidic devices.

Several studies have shown how electric fields can be used to discriminate cell depending on their dielectric properties, which represents a growing interest for many biomedical applications (target cell identification from an heterogeneous biological sample, different stages of cancer disease diagnosis,...).

This paper presents a microfluidic device devoted to the determination of a single cell electro-physiological properties combining dielectrophoresis force for the cell trapping, with electrorotation experiments to extract the dielectric properties of the cell. A microfluidic device has been designed for this purpose and evaluated with two different cell lines.

Combining dielectrophoresis and electrorotation experiments allows reproducible measurement by avoiding possible perturbations or interactions with other cells during the analysis within the biodevice. External solicitations applied to biological cell might be monitored in 'real-time' in such microfluidic platform.

1. INTRODUCTION

Electric fields as a way to interact with bioparticles (cells, bacteria, algae, etc.) are widely used in microfluidic devices [1][2][3]. Several studies have shown how it is possible to use the electric field to separate cells via their intrinsic characteristics and, by consequence, characterize them from their electro-physiological features [4]. The underlying phenomenon is the contrast between the cell and medium dielectric polarizations.

From an electrical point of view, an eukaryotic cell behaves as a highly conducting cytoplasm delimited by an insulating membrane surrounded by the extracellular medium. For the circulating cell case, cell appears as a spherical particle (radius close to 7 μm) having a thin membrane (d=8 nm) which separate the inner compartment from the outer compartment. A common approach leads to consider the cell as an homogenised particle with an equivalent complex permittivity (ϵ^*) estimated from the multi-shell model, or the single shell model [5]. In the latter case the cell nucleus is not considered.

Once immersed in an external electric field, the cell polarizes. The interaction between its dipolar moment and the electric field generates forces that allows the cell handling [6]. When a non uniform electric field is applied, dielectrophoresis force is generated and results in a movement of the cell which is attracted by the highest or lowest EF area (respectively pDEP or nDEP case) depending on the polarizability of the equivalent particle with respect to the polarizability of the suspending medium [7].

Besides, a cell immersed in a rotating electric field that is non uniform in phase, experiences a rotational movement due to the interaction between the induced dipole and the rotating field. From the analysis of the electrorotation spectrum, the estimation of the electro-physiological properties of cells can be performed [8][9]. Such determination of the cell dielectric properties is an efficient mean to characterise cell's viability, type, or simply susceptibility to respond to positive or negative DEP for further sorting or separation purpose within a microfluidics device [10][11][12].

Our work is based on the use of trapping conventional dielectrophoresis forces thanks to creation of a stationary electrical field in order to precisely locate cell at the center of the analysis chamber, prior to superimposed a rotating electric field provoking the cell electrorotation (Fig.1a). The rotational torque $\Gamma(\omega)$ applied on the cell is directly linked to the cell properties and it is calculable through [9]:

$$\Gamma(\omega) = -4\pi r^3 \epsilon_m \text{Im}[f_{CM}(\omega)] E_{rms}^2 \quad (1)$$

$$f_{CM}(\omega) = \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*} \quad (2)$$

r represents the cell radius, ϵ_m the medium permittivity, $\text{Im}[f_{CM}(\omega)]$ the imaginary part of the Clausius-Mossotti factor ($f_{CM}(\omega)$) and it is dependent upon complex permittivity of the media and cell composition (Eq. 2 - respectively ϵ_m^* and ϵ_p^*).

Taking into account also the effect of the medium viscosity (η), the cell rotates with the following velocity $\Omega(\omega)$:

$$\Omega(\omega) = \frac{\Gamma(\omega)}{8\eta\pi r^3} = -\frac{\epsilon_m \text{Im}[f_{CM}(\omega)] E_{rms}^2}{2\eta} \quad (3)$$

Depending on the value (sign and amplitude) of $\text{Im}[f_{CM}(\omega)]$, an electrorotation spectrum can be generated and evaluated, reflecting the individual electrical properties of cells. An example of a theoretical electrorotation spectrum for Jurkat cell line suspended in an isotonic medium ($\sigma_m=0.1\text{S/m}$) is given in Figure 1b with classical referred dielectric properties.

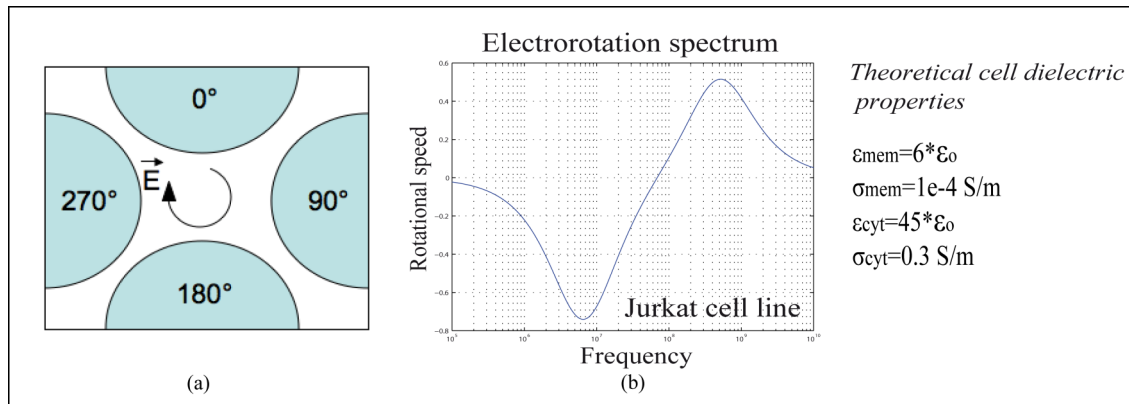


Figure 1: (a) Electrode shape for rotating field induction. (b) Theoretical electrorotation spectrum.

To study cell behavior undergoing electric field forces, the equivalent particle (ϵ_p^*) is calculated with the single shell model [5]. This model is deduced from the cell shape and composition :

$$\epsilon_p^* = \epsilon_{mem}^* \frac{\left(\frac{R_p}{R_p - e}\right)^3 + 2 \left(\frac{\epsilon_{cyt}^* - \epsilon_{mem}^*}{\epsilon_{cyt}^* + 2\epsilon_{mem}^*}\right)}{\left(\frac{R_p}{R_p - e}\right)^3 - \left(\frac{\epsilon_{cyt}^* - \epsilon_{mem}^*}{\epsilon_{cyt}^* + 2\epsilon_{mem}^*}\right)} \quad (4)$$

$$\epsilon_x^* = \epsilon_0 \epsilon_x - j \frac{\sigma_x}{\omega} \quad (5)$$

Where ϵ_p^* is the equivalent permittivity of the particle, R_p the cell radius, e the thickness of the cell

membrane, ϵ_{cyt}^* is the complex permittivity of the cytoplasm (intracellular medium with permittivity ϵ_{cyt} and conductivity σ_{cyt}), surrounded by a lipid bilayer characterized by its complex permittivity ϵ_{mem}^* (with permittivity ϵ_{mem} and conductivity σ_{mem}).

Once the electro-physiological fingerprint of the cells is extracted, this method might be used as a tool to distinguish malignant cells from healthy ones, based on their dielectric properties [13], or even to discriminate different types of cells, bacteria, yeasts, for further sorting [14].

In this paper we present results obtained in a microfluidic device designed to determine the electrophysiological properties of a single cell combining a stationary wave dielectrophoresis for the cell trapping, and an electrorotation force for the parameters estimation. It had been applied to two different cell types classically used as cellular model in biology.

This method might be used for other applications such as the electropermeabilization monitoring. Actually the knowledge of the dielectric properties of the cell before and after pulsed electric field (PEF) application is of primary importance in order to determine the proper PEF conditions to permeabilize cell in a reversible manner for instance for gene or drug electro transfer [15][16]

2. MATERIALS AND METHODS

2.1 Sample preparation

Our experiments were performed on two different cell lines: Jurkat E6.1 and B16F10. Jurkat cells (human leukemic T cell lymphoblast) are immortalized line of human T lymphocyte, we used the clone E6.1 (acute T cell leukemia) which was grown in RPMI 1640, where bovine fetal serum (10%) and penicillin streptomycin (1%) were added. The cells were collected by centrifugation and re-suspended in an isotonic medium ($\sigma_m=0.1\text{S/m}$) prepared for the electrorotation experiments. The same protocol was employed for B16F10 cell line, murine melanoma cells which present an increased rate of generation of metastatic variants. This alteration suggests a gene amplification mechanism that may be involved in the expression of metastatic phenotype. Furthermore they are resistant to a given concentration of chemotherapy drugs.

To synchronize cells to the phases G0 and G1, a culture medium with small percentage of Fetal Bovine Serum (FBS=2% of the total volume) was prepared, cells were treated subsequently with the normal culture medium (FBS=10% of the total volume) and then with the new one. The treatment employed interferes with the normal cell growth since the new medium (low FBS percentage) is able to slow down the cell division and thereafter to block cell in the first two phases of the cell life cycle.

2.2 Biochip design

The electrorotation and dielectrophoresis experiments were performed on a dedicated biodevice with four planar electrodes arranged as showed in Fig.2a. 3D finite element simulation (COMSOL ©) was implemented to study different electrodes shapes (Fig.2a-b). The aim was to determine the optimized structure that both induced the largest homogeneous electric field (EF) area in the centre of the biochip (Fig.2c-d) and rejected cells that may come into the interesting area thus interfering with the cell analysis.

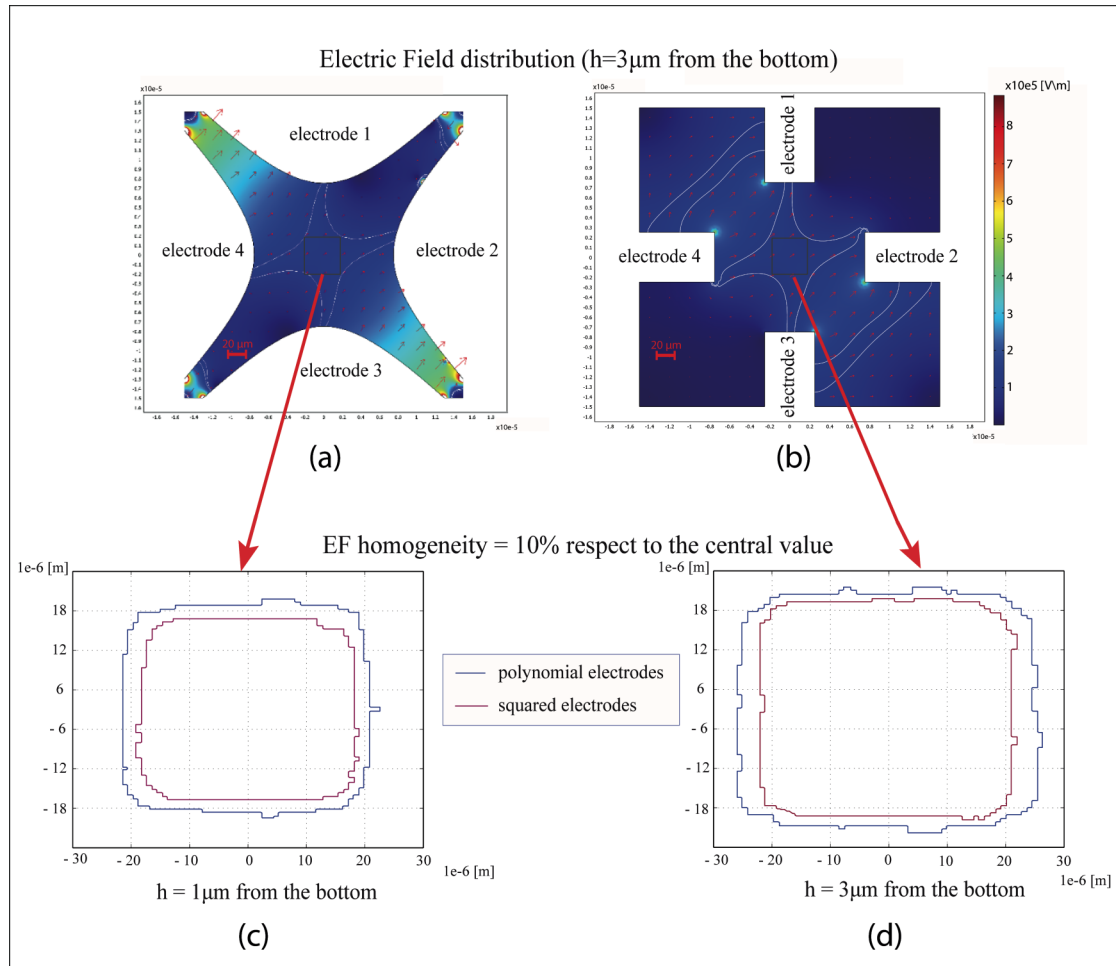


Figure 2: (a-b) EF distribution for two different electrode shapes with a field angle of 45° . (c-d) EF spatial homogeneity in the centre of the biodevice at different planes for all field angles.

Various parameters such as shape, size and distance between electrodes have been tested. We took into account the spatial distribution of the electric field and its time evolution during signals application. A large area presenting a satisfactory uniformity of the electric field is required in order to avoid shifting of cells during the measurement.

The choice of the optimized electrode shape was done by studying the distribution of normalized value of electric field for field angles; in a first approach we simplified the problem by evaluating the field along two main directions (defined by vertical or horizontal and 45° inclination). The polynomial shape showed the best performance in terms of largest homogeneous EF area (Fig.2c-d). Furthermore in our experiments cells were exposed to a negative dielectrophoresis and the polynomial shape induced a high EF in the peripheral area, thus every possible disturbance (other cells) could be repulsed from the central area of the biodevice where a target single cell was studied.

2.3 Biochip fabrication

The chip has been fabricated using standard clean room processes. Starting from a quartz substrate, covered with a 20 nm chromium adhesion layer followed by a 150 nm thin gold layer (Fig.3a), a device with four planar polynomial gold electrodes had been fabricated with a distance of 75 μm from face to face electrodes. The process includes a 30 s spin coating of S1805 Shipley photoresist at the speed of 1000 rpm, a prebake at 115°C for 1 min. A photolithography of 8 s with intensity equal to $16\text{ mW}/\text{cm}^2$ was then done to obtain, after wet etching of gold and chromium layers, the desired electrode shape (Fig.3b-c). The microfluidic channels were defined with a thick SU8 layer (30 μm) in a second photolithography step including spin coating in 2 steps ($t_1=5\text{ s}$, $v_1=500\text{ rpm}$ and $t_2=30\text{ s}$, $v_2=2700\text{ rpm}$) with SU8 2025 and a soft baking (3 min at 65°C , 12 min at 95°C) before the photolithography using UV illumination (intensity= $16\text{ mW}/\text{cm}^2$, $t=18\text{ s}$). Then a post exposure bake was done (3 min at 65°C , 15 min at 95°C) and followed by the development step (MicroChem SU8 developer, $t=6\text{ min}$) to get the microchannel surrounding the electrodes (Fig. 3d).

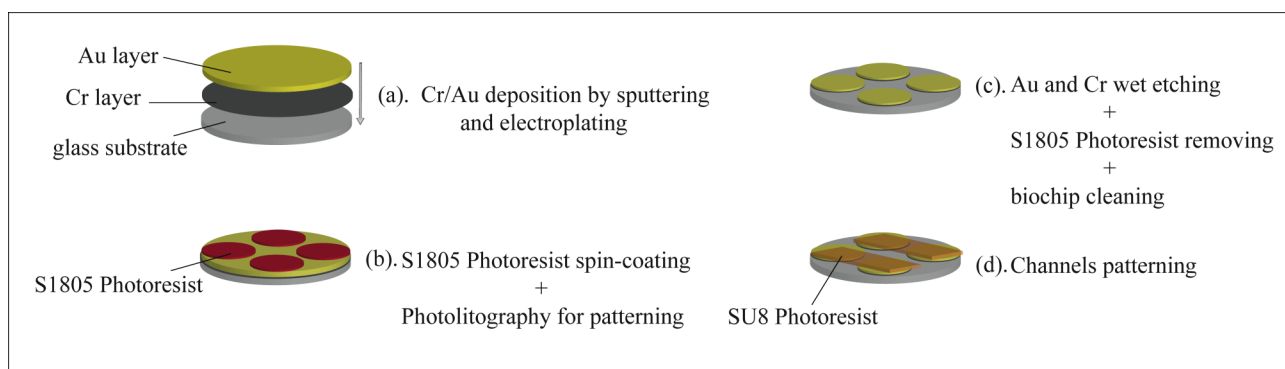


Figure 3: Biodevice microfabrication process.

2.4 Electric field management : trapping and rotation

The electric field is obtained with four electrodes, each connected to a synchronized voltage generator. Depending on the topology needed (Trapping or Rotation), the four voltages (V_a , V_b , V_c , V_d) are associated to the same frequency but with a different phase (Fig.4).

Dielectrophoretic forces for the cell trapping were induced by a stationary electrical field generated from two sinusoidal signals of opposite phase (amplitude 5Vpp, frequency 300kHz). At this frequency the cell, undergo negative dielectrophoresis [17], was trapped in the minima of electrical field at the centre of the biodevice, between the four electrodes (see Tab.1).

Once the cell was trapped, a superposed rotating torque was applied (Fig.4a). The torque was generated by a rotating propagative electric field relying on four sinusoidal signals with the same amplitude (2Vpp), having 90° phase difference from each other in a wide frequency range [100kHz-20MHz] (see Tab.1). The torque depends on the electrical field amplitude, the medium electric properties as well as the imaginary component of the Clausius-Mossotti factor $\text{Im}[K(\omega)]$, which is related to the cell dielectric properties .

Rotation of cells was acquired with a high-speed camera. After image analysis, an electrorotation spectrum was obtained (mechanical velocity of the cell with respect to the electrical frequency of the applied voltages). The experimental setup includes a customized interface made under Labview™ for signal generator remote control (Fig.4b).

The system proposed is thus capable to trap a single cell in the middle of the four electrodes by avoiding any disturbance caused by other cells which are rather repelled from the central area. Thus the measurement is preserved and it can be performed with another cell with unchanged applied conditions.

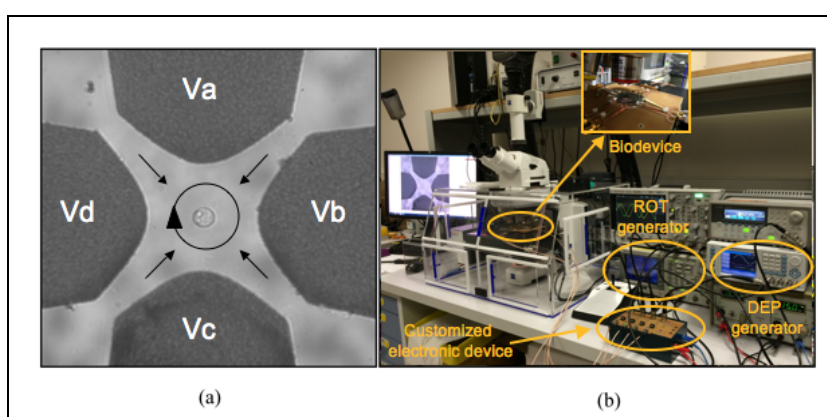


Figure 4: (a) Cell submitted to both nDEP and electrorotation. (b) Experimental system.

	V_a	V_b	V_c	V_d
DEP signals (cell trapping)	0°	180°	0°	180°
ROT signals (cell rotation)	0°	90°	180°	270°

Table 1: Electrode's potentials decay of phase management for forces superimposition.

2.5 Parameters estimation

The cell dielectric parameters (complex permittivity of cell membrane and cytoplasm) were determined from the electrorotation spectrum curve using a customized MatlabTM program. Determination of electro-physiological parameters of cell is a classical study of “parameters estimation” where mathematical structure of theoretical curve is known and unknown parameters are calculated by using experimental data [18].

The trust-region-reflective algorithm which is part of the Gauss-Newton algorithms was chosen for its reliability and its robustness, it is well used to solve ill-conditioned problems thanks to its very strong convergence properties [19].

The program uses theoretical values of electric parameters of particle (such as membrane/medium permittivity and conductivity respectively, ϵ_{mem} ϵ_m , and σ_{mem} and σ_m) and draws a theoretical electrorotation spectrum. From experimental values of cell rotating speed, we generate an experimental rotation spectrum. This two obtained spectra, theoretical and experimental, are used to get an optimized spectrum throughout a least square algorithm [20].

3. RESULTS

The biochip was tested and validated with two cell lines, B16F10 and Jurkat ones re-suspended in an isotonic medium ($\sigma_m=0.1S/m$), in the frequency range of 10kHz up to 20MHz.

In order to simplify the experiments, the channel was just covered by a glass slide, which simplified the cleaning and re-use of the bio-device. A small volume of cells in the suspending medium (about 30 μ l) was dropped on the microfluidic device in correspondence to the centre of the four electrodes. The drop was thus covered by a thin glass slice to obtain the microfluidic channel. (Fig.5a)

Once the cell is captured in the centre between the four electrodes by negative dielectrophoresis, a rotating electric field was applied and thereby a rotational movement was induced. The dielectric properties of cell, both membrane and cytoplasm conductivity and both membrane and cytoplasm permittivity, were thus estimated (Fig.5b). Extracted parameters are summarized in the Tab.1.

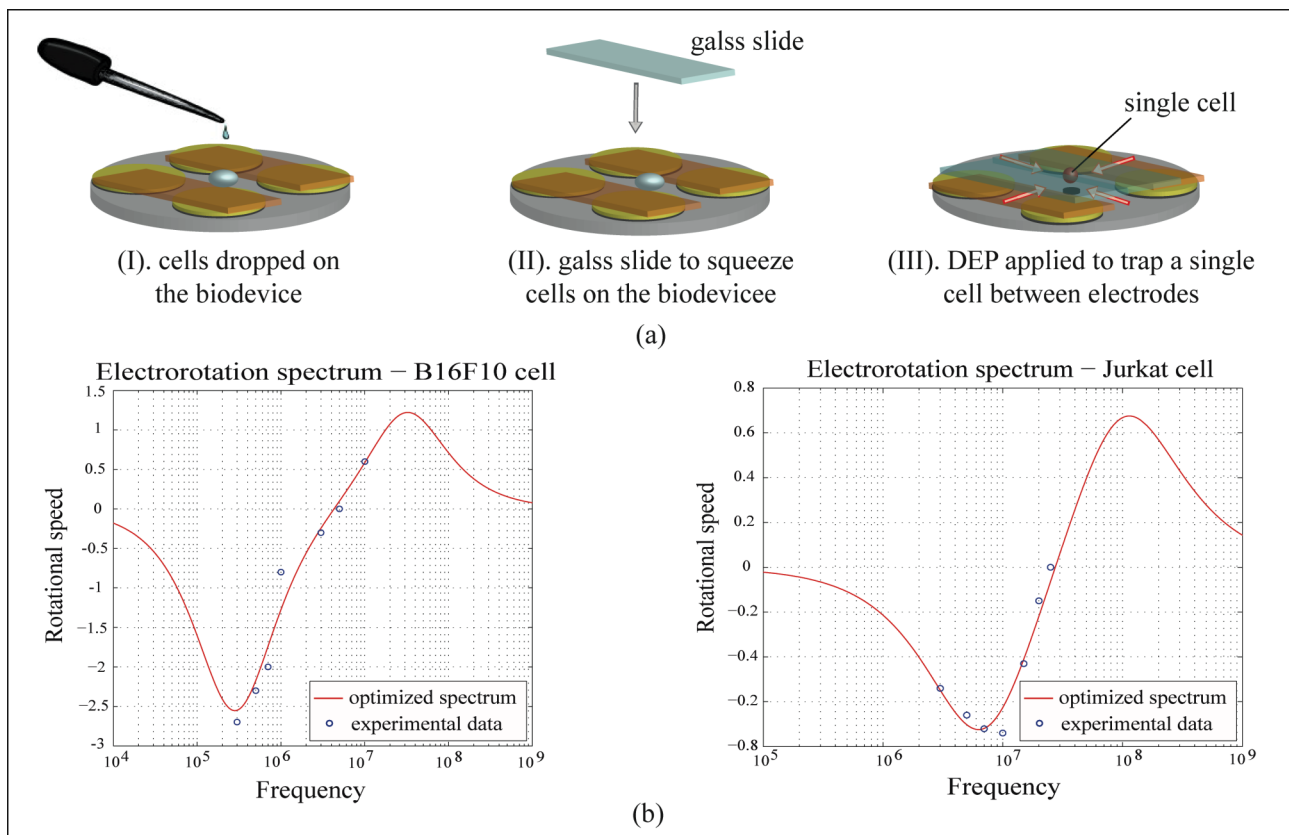


Figure 5: (a) Procedure to treat cells with the biodevice. (b) Electrorotation spectrum of B16F10 cell line (on the left) and Jurkat cell line (on the right), cell were in both cases synchronized in their lifecycle.

	$\epsilon_{\text{mem,rel}}$	$\sigma_{\text{mem}}[\text{mS/m}]$	$\epsilon_{\text{cyt,rel}}$	$\sigma_{\text{cyt}}[\text{S/m}]$
Estimated value B16F10 cell line	6	0.01	44	1.7
Estimated value Jurkat cell line	1	0.015	40	0.58
Theoretical values (B16F10)	6	0.1	45	0.3

Table 1: Estimated dielectric properties of cell by electrorotation analysis.

Such estimated parameter are in the same order than the one found in the literature (cf. Tab.1)[21].

The technique presented in this paper, combining trapping dielectrophoresis and electrorotation, is promising. Further experiments are under investigation in order to monitor the change of cell dielectric properties once submitted to various sollicitations (experimental medium conductivity, medium osmolarity, thermal or electrical external applied sollicitations).

Working with the same applied conditions, the experiments performed without cells lifecycle synchronization resulted in different rotational speed for cells within the same sample, whereas for synchronized cells rotational velocity revealed to be almost unchanged for cells within the same sample. We actually got for five different synchronized cells a rotational velocity with an error that varies from 6% in the best case to 13% in the worst case, the difference in rotational velocity being caused by the fact that cells were in different phases of their lifecycle.

4. CONCLUSION

This work presents a combined electrorotation and dielectrophoresis technique to determine dielectric properties of a single cell. The dielectrophoretic force is first applied to trap the cell in the center of the four-set-electrodes structure, followed by a rotational torque which is superposed in order to induce electrorotation of the cell. The automated control of the whole system (signal generators and image acquisition) is based on a Labview © interface which synchronizes the data acquisition to the frequency sweeping for electrorotation. The rotational velocity of cells was observed and performed successfully on a wide range of frequencies for two different cell lines (B16F10 and Jurkat) synchronized on their lifecycle.

The use of this platform for the real-time monitoring of the cell dielectric properties during their electropermeabilization is under investigation. Indeed the efficiency of drug delivery using electropermeabilization protocols might be characterized using such approach.

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